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(19) (CA) **CANADIAN PATENT** (12)

(54) 9-(Aminoalkyl)-8-Hydroxyadenines and Method of Their Preparation

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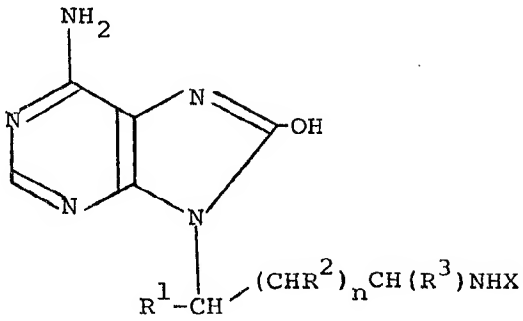
ABSTRACT OF THE DISCLOSURE:

The present invention relates to new compounds of general formula



(I)

where R^1 and R^3 is hydrogen, alkyl, or the hydroxyalkyl group, R^2 hydrogen or the hydroxy group, X is H or $CO(CH_2)_6R^4$, n is 0 or 1, and R^4 is the residue of a suitable support e.g. a macromolecular dextran gel. The present invention also provides a method of preparation of these compounds, i.e. of 9-(aminoalkyl)-8-hydroxy-adenines or derivatives thereof. The above compounds may serve as ligands for the preparation of modified polymerous dextran gels for affinity chromatography of S-adenosyl-L-homocysteine hydrolase.

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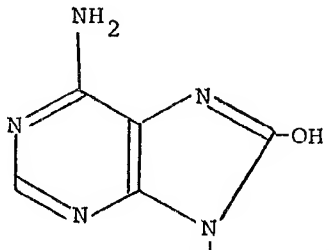
The present invention relates to new compounds of general formula I



(I)

where R^1 and R^3, independently, are a hydrogen atom, an alkyl group , an hydroxyalkyl group , R^2 is hydrogen or an hydroxy group, X is H or is the residue of a suitable support, n is 0 or 1. X may be $CO(CH_2)_6R^4$ where R^4 is the residue of a macromolecular dextran gel. The support may be organic or inorganic.

Alkyl groups or moieties thereof may have from 1 to 4 carbon atoms, i.e. for R^1 and R^3.

An efficient purification and isolation of enzymes can be carried out by the so-called affinity chromatography based on strong specific and reversible bonding of the enzyme to a polymer support with immobilized low molecular weight ligands having the character of modified substrates, products, or inhibitors of the corresponding enzyme.

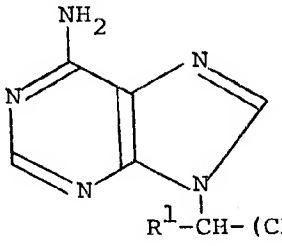
One of the important enzymes which controls the basic metabolic functions of the living organism is S-adenosyl-L-homocysteine hydrolase (SAH-hydrolase). This enzyme is present in all kinds of eukaryotic cells and its level may be related to the development of some metabolic disorders. Data on the relationship between this enzyme and the growth of experimental tumors in animals have been

reported (G.N. Orlov, J.V. Bukin: Voprosy med. chim. 26, 699 (1980)). This finding stimulated interest in methods of isolation, purification, and determination of the level of this enzyme in samples of biological material. The SAH-hydrolases are very unstable and loose their enzymatic activity in the course of procedures routinely used for the isolation of proteins (fractionation by precipitation, ion exchange chromatography, gel filtration). Methods which permit a rapid and specific isolation of these enzymes from crude biological extracts are the most suitable ones; such a method is, e.g. affinity chromatography.

Affinity chromatography as a method of isolation of SAH-hydrolase on a support with immobilized 8-(3-aminopropylamino)adenosine has been reported (E.O. Kajander, A.M. Raina: Biochem. J. 193, 503 (1981)); this method, however, is not very efficient. A procedure based on the use of polymer supports with covalently bonded 9-(RS)-(3(2)-aminopropylamino-2(3)-hydroxypropyl)-8-hydroxyadenine (a.c., PV 8920-82) has also been described; this procedure is very efficient yet it requires a ligand which can be prepared by a method which is technically relatively demanding (a.c. PV 8919-82).

These drawbacks can be eliminated by using the subject of this invention, the new compound of formula I described above.

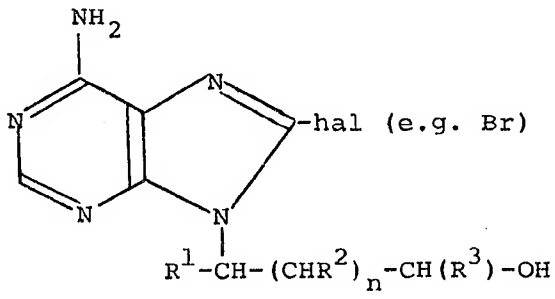
The present invention also relates to a method of preparation of compounds of general formula I, where X is H(9-(aminoalkyl)-8-hydroxyadenine). According to this method 9-(hydroxyalkyl)adenine of general formula II,



(II)

R^1–CH–$(CHR^2)_n$CH(R^3)OH

where R^1 through R^3 and n are the same symbols as in formula I, is allowed to react with a halide (e.g. bromine) in aqueous solution or suspension at a temperature of 0 to 30°C and the 9-(hydroxyalkyl)-8-bromoadenine formed of general formula III,

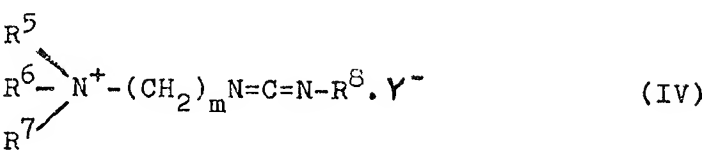


(III)

where R^1 through R^3 and n are the same symbols as in formula I, is heated with an aqueous 20 to 30% (by weight) solution of ammonia at a weight ratio of 1:10 to 1:40 at a temperature of 80 to 150°C.

The present invention also relates to a method of preparation of compounds of general formula I, where X is $CO(alk)R^4$, alk being a suitable alkylene chain e.g. $(CH_2)_6$ and R^4 is the same symbol as in formula I above. According to this method a dextran gel bearing an ω-carboxyalkyl, to advantage a 6-carboxyhexyl group, is allowed to react with 9-aminoalkyl-8-hydroxyadenine of general formula I, where X is H, the ratio being 1 to 10 molar equivalents per one carboxyl group of the dextran gel, and with salts of soluble

carbodiimides of general formula IV,



(IV)

where R^5 through R^7 are methyl groups, or R^5 and R^6 are together $(-CH_2CH_2)_2O$ and R^7 the methyl or ethyl group, R^8 the cyclohexyl or ethyl group, Y^- the chloride or p-toluenesulphonate anion, and m is 1 to 4. The reaction is allowed to proceed in an aqueous solution at pH 5 to 6 and temperatures of 0 to 30 °C and after completion of the reaction the insoluble gel is filtered off and washed with water and solutions of neutral buffers.

Still another subject of the invention is the use of the affinity support according to the invention for the isolation of S-adenosyl-L-homocysteine hydrolases from extracts of biological materials, consisting in the adsorption of the crude or partly purified extract of the biological material either batchwise or in a column to the affinity support of general formula I, where X is $CO(CH_2)_6R^4$; after washing of the support with aqueous buffered solutions of neutral electrolytes the S-adenosyl-L-homocysteine hydrolases are specifically displaced by a small volume of 0.01 to 1.0 $mmol.l^{-1}$ adenosine solution in an aqueous buffer at temperatures of 0 to 20 °C and subsequently adenosine is removed from the effluent by gel filtration or dialysis.

The advantage of the preparation of compounds of formula I, where X is H, according to the invention consists in the fact that the replacement of the substituent in position 8 of

the compounds of formula III and the exchange of the hydroxyl group in their side chain for an amino group may proceed in one reaction step and under extraordinarily simple conditions. The condition necessary for this reaction to proceed is the possibility of formation of cyclic intermediates (A.Holý, Collect.Czech.Chem.Commun. 48, 1910 (1983)) and at the same time is limited to the preparation of such compounds of formula I, where X is H, in which the amino group of the side chain is in alpha or beta position with respect to the carbon atom binding the adenine ring.

The starting compounds of general formula II are generally well accessible, e.g. by alkylations of adenine (A.Holý, Collect.Czech.Chem.Commun. 43, 3103 (1978); 43, 3444 (1978); 43, 2054 (1978); 44, 593 (1979); 47, 173 (1982)). The reaction of these compounds with bromine easily proceeds in an aqueous medium, both homogeneous and heterogeneous, with a small excess (20 to 50%) of bromine and yields hydrobromides of formula III as the only reaction products. These compounds can be reacted with ammonia directly; it is, however, advantageous to isolate the compounds of formula III thus eliminating the hazard of formation of colored contaminants in the products which are removable with difficulties only. This isolation is carried out after evaporation of water from the reaction mixture in vacuo (e.g. 2 to 2.5 kPa at temperatures of 30 to 50 °C) by careful and exact neutralization of the reaction mixture with concentrated (0.5 to 4.0 $mol.l^{-1}$) aqueous solutions of alkaline hydroxides (with lithium hydroxide to advantage) thus precipitating the very little soluble product of formula III in most cases. If compounds of formula III are more or less

soluble in water they can be easily extracted from the dry evaporate of the neutralized reaction mixture with chloroform or its mixtures with methanol or ethanol; they can be isolated from the neutralized solution of the reaction mixture to advantage also by deionization on a cation exchanger which is washed with water and subsequently weakly alkalized by the addition of a volatile amine (ammonia to advantage) and the compound of formula III is obtained after evaporation of this effluent.

The reaction of the compounds of formula III is carried out to advantage by heating with concentrated aqueous ammonia, in suspension or solution, without external stirring. Since the pressure in the closed reaction vessel does not rise to high values at the temperatures used, simple common, low-pressure reaction vessels, resistant to aqueous ammonia, or glass thick-walled reactors can be used for the reaction.

The control of the course of the reaction and of the purity of the reaction products is carried out to advantage by paper chromatography or thin-layer chromatography on silica gel, in both cases in the system 80% aqueous 2-propanol-concentrated aqueous ammonia (9:1); the detection is performed in ultraviolet light and the compounds of formula I, where X is H, are detected by spraying the chromatogram with ninhydrin (violet spots).

The reaction yields generally compounds of formula I, where X is H, as the only reaction products. The evaporation of the reaction mixture affords a mixture of the product or its hydrobromide with ammonium bromide. The compound of formula I, where X is H, can be obtained in pure state by chromato-

graphy, e.g. on silica gel or cellulose in the mixture 80% aqueous 2-propanol-con. aqueous ammonia (9:1) or by chromatography on octadecylsilica gel in water.

The binding of compounds of formula I, where X is H to suitable supports may be effected by any suitable mechanism, e.g. between the amino group and a suitable reactive functional group on a suitable (polymer) support, e.g. carboxyl group, acylhalide group, aldehyde group, etc.

The binding of compounds of formula I, where X is H, to polymer supports may, for example, be effected by formation of an amide linkage, i.e. an amide bond between the compound and the carboxyl functions of the polymer support. Such a support may be a dextran gel (e.g. Sepharose, Agarose) and also cellulose or other polymer material which has been modified in advance to contain free carboxyl groups attached to the polymer matrix through sufficiently long, e.g. polymethylene (hexamethylene) chains. Since the amide bond formed is chemically stable and resistant to the action of most enzymes the polymer materials thus prepared of formula I, where x is $CO(CH_2)_6R^4$, are stable for several months at temperatures of 0 to 10°C in water or, to advantage, in saturated solutions of sodium or potassium chloride. The reaction in the presence of so-called soluble carbodiimides of general formula IV, by which the condensation is effected, takes place under mild conditions which do not deteriorate the structure of the polymer matrix.

The polymer supports of formula I, where x is $CO(CH_2)_6R^4$, show a high affinity for SAH-hydrolases. They can be used therefore for specific concentration of these enzymes during their isolation from various crude or only partly purified extracts of various biological materials. Thus, e.g. compounds of formula I, where x is $CO(CH_2)_6R^4$, can be added to advantage to a dilute solution till its enzymatic activity disappears; subsequently the polymer support is washed to remove contaminating proteins and other components not adsorbed (dyes

etc.) with solutions of neutral electrolytes of increasing ionic strength, to advantage at 0 to 10 °C. The SAH-hydrolase is subsequently eluted from the support by dilute solutions of adenosine which is a substrate or, alternatively, an inhibitor of this enzyme. The consumption of adenosine for this purpose is minimal. The whole isolation procedure can be carried out equally well batchwise with filtration after each elution step or on a column of the above support. Adenosine is removed from the effluent containing the purified enzyme by dialysis, ultrafiltration, or gel filtration (e.g. on Sephadex ® or Biogel ®).

This procedure of isolation of SAH-hydrolases is not very demanding as regards time and material and yields very pure, entirely or almost entirely homogeneous proteins (according to gel electrophoresis). It is therefore especially suited for rapid analyses of biological materials, tissue extracts etc. in those cases where a small quantity only of the preparation is available which could not be obtained by other isolation procedures. The procedure can be also used for preparative-scale isolations when larger quantities of the homogeneous proteins are to be isolated.

S-Adenosyl-L-homocysteine hydrolase is of considerable preparative importance from this viewpoint: using this enzyme S-adenosyl-L-homocysteine can easily be prepared from adenosine and L-homocysteine, at low cost in an aqueous medium in a high yield, with the possibility of adenosine regeneration (Chabannes B., Charit A., Cronenberger L., Pachéco H.: Prep. Biochem. 12, 195 (1982)). Since S-adenosyl-L-homocysteine is the starting material for S-adenosyl-L-methionine used as

a drug, a simple purification of SAH-hydrolases, especially the removal of contaminating enzymes degrading adenosine, is of technical importance.

The general procedure of compounds of formula I according to the invention is given below and their use for the isolation of SAH-hydrolases is illustrated by additional examples which in no way limit its scope.

EXAMPLE 1:

The solution of 1 ml of bromine in 150 ml of water is treated with 10 mmol of compound of formula II and the mixture is stirred in a closed vessel at a temperature of 18 to 24° C for 16 to 24 h. The suspension is then evaporated at 40° C/kPa to dryness and the residue is dissolved in 100 ml of water. The solution is neutralized to pH 7.0 (6.95 to 7.05) with stirring using a pH-meter and 4 $mol.l^{-1}$ sodium or lithium hydroxide and subsequently cooled down to 0° C for 1 to 2 h. The separated product of formula III is filtered off, washed with water (200 ml), acetone (100 ml) and ether (100 ml), and dried at 10 to 15 Pa over phosphorus pentoxide. The yields and properties of compounds of formula III prepared by this procedure are given in Table 1 at page 15.

The suspension of 5 mmol of the compound of formula III in 50 ml of concentrated aqueous ammonia (25 to 29% NH_3) is heated in a steel pressure vessel at 100 to 110° C for 2 to 8 h. After cooling the clear pink solution is evaporated at 40° C/2 kPa to dryness, the residue is dissolved in 20 ml of a mixture of 80% aqueous 2-propanol and conc. aqueous ammonia (9:1) and applied to a column (80 x 4 cm) of microcrystalline

cellulose in the same system. The column is eluted (at a rate of 20 ml/h) by the same system and the fractions (20 ml) are analyzed by paper chromatography in the same system. The product-containing fractions are pooled, taken to dryness at 40 °C/2 kPa, the residue is evaporated with ethanol (2 x 20 ml) under the same conditions, and the residue is crystallized from methanol with the addition of ether till turbidity appears. The obtained compound of formula I, where X is H, is filtered off, washed with ether, and dried at 10 to 15 Pa over sodium hydroxide or natron calk. The mother liquors contain an additional amount of the compound in the form of bicarbonate which can be repeatedly subjected to the same chromatography or precipitated with ether and isolated as the bicarbonate. The yield and the properties of the compounds thus prepared of formula I, where X is H, are given in Table 2 at page 16.

Example 2

The compound of formula II (10 mmol) is reacted with bromine and treated as described under Example 1. In case that the product of formula III is water-soluble and does not precipitate from water the following procedure is used:

A neutral aqueous solution of the dry residue of the reaction mixture is applied to a column (200 to 250 ml) of a cation exchanger (Dowex® 50 to advantage) in acid form and the column is washed with 1 l of water. The ion exchanger is then suspended in 400 ml of water and treated with stirring with dilute (1:2, vol/vol) aqueous ammonia so that the pH is kept below 8 until this value remains constant for 30 min. The suspension is filtered off and the ion exchanger is washed

with boiling water (0.5 to 1.0 l). The filtrate is evaporated at 40° C/2 kPa and the dry residue is crystallized from ethanol or 80% aqueous ethanol. The product is filtered off and dried at 10 to 15 Pa over phosphorus pentoxide. The properties and yields of the compounds thus prepared of formula III are given in Table 1 at page 15. The subsequent procedure is the same as that described under Example 1.

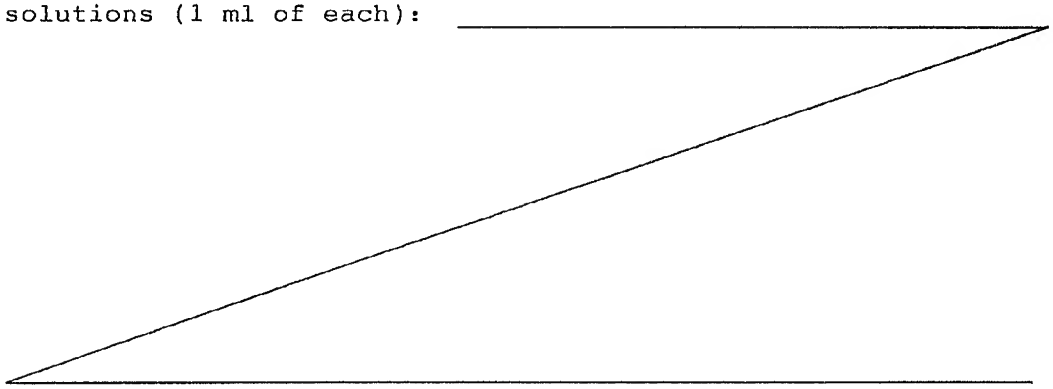
EXAMPLE 3:

The dextran gel slurry (200 ml), e.g. CH-Sepharose (a registered trade mark of the product of Pharmacia, Uppsala, Sweden), where CH stands for the gel with modified carboxylhexyl groups) is washed stepwise with 0.1 $mol.l^{-1}$ sodium bicarbonate (5 litres) and water (4 litres). The compound of formula I, where X is H, is added to a suspension of 10 ml of the gel slurry in 30 ml of water (usually a two- to three-fold excess in terms of the carboxylate capacity of the support). The pH of the mixture is adjusted to pH 5.0 by 2 $mol.l^{-1}$ hydrochloric acid with magnetic stirring using a pH-meter and the first portion is added of the compound of formula IV (usually a 2.5-fold excess with respect to the compound of formula I, where X is H). The suspension is stirred and the pH is maintained at 5.0-5.5 by hydrochloric acid (2 $mol\text{-}l^{-1}$). The same portion of the compound of formula IV is added 30 min later and the pH of the mixture is maintained in the same manner until it remains constant. The pH is adjusted to 5.0 and the suspension is gently shaken for 15 to 24 h at room temperature (18 to 25° C). Subsequently the suspension is filtered off, the gel is washed with water (500 ml) and suspended in 20 ml of 0.5 $mol.l^{-1}$ solution of 2-aminoethanol hydrochloride (pH 5.0). Subsequently the third, same portion of the compound of formula IV is added and the pH is again maintained at 5.0-

5.5 by adding 2 mol.l^{-1} hydrochloric acid. The suspension is gently shaken for additional 3.5 h, filtered off afterwards, and washed stepwise with water (1 liter) and saturated solution of potassium chloride. The support thus prepared of formula I, where X is $CO(CH_2)_6R^4$, is stored in saturated solution of potassium chloride containing 0.02% of sodium azide at a temperature of 0 to 4° C. Specific data on the affinity support types of formula I, where X is $CO(CH_2)_6R^4$, obtained in this manner and on the mutual ratios of reactants used are given in Table 3 at page 17.

EXAMPLE 4:

The isolation of SAH-hydrolase from rat liver is carried out with a partly purified concentrate of this enzyme (Votruba I., Holy, A.: Collect. Czech. Chem. Commun. 45, 3039 (1980)) in 0.1 mol.l^{-1} sodium chloride having a specific activity of 0.9 EU (EU stands for international units of enzyme activity) per mg of protein. This enzyme preparation is diluted 1:4 with 0.01 mol.l^{-1} Sörensen potassium phosphate buffer, pH 7.37, containing 0.001 mol.l^{-1} dithiothreitol. The whole procedure is carried out at 0° C. The support (0.6 ml) of formula I, where X is $CO(CH_2)_6R^4$, is added to 1 ml of the dilute enzyme solution and the suspension is gently shaken 20 min at 0° C; it is then centrifuged and the sediment shaken stepwise for 20 min with the following solutions (1 ml of each):



0.01 mol.l^{-1} Sörensen phosphate buffer, pH 7.37, same buffer yet 0.2 mol.l^{-1}, 1 mol.l^{-1} potassium chloride, 1.5 mol.l^{-1} potassium chloride. 2.0 mol.l^{-1} potassium chloride, 0.025 mol.l^{-1} adenosine in 0.75 mol.l^{-1} potassium chloride. All buffers contain 0.001 mol.l^{-1} dithiothreitol. The enzymatic activity of SAH-hydrolase is determined in all eluates by the procedure described under Example 5. The individual eluates are obtained by centrifugation of the suspension. The properties of the supports used in this example and the yields of SAH-hydrolase isolated from the last eluate are summarized in Table 4 at page 18.

Example 5

The wet mass (100 g) of a suspension culture of Nicotiana tabacum cells is washed on a glass filter with 0.01 mol.l^{-1} potassium hydrogenphosphate, pH 7.4 (2 liters) and the pellet is triturated in liquid nitrogen in four portions with 15 g each of Al_2O_3 (alumina 305). The material obtained is suspended in 200 ml of the same buffer also containing 1 mmol.l^{-1} dithiothreitol and 14 g of polyvinylpyrrolidone. The extract is centrifuged 40 min at 27 000 g and the supernatant is treated with slow stirring at 4°C with ammonium sulfate added to 80% saturation. The suspension is centrifuged 25 min at 30 000 g and then dissolved in the above buffer containing dithiothreitol (100 ml). A total volume of 10 ml of the affinity support (No 3, Table 3) is added successively with stirring. The mixture is stirred 30 min at 30 °C, filtered off and then washed stepwise with 40-ml portions of the first five washing buffers described under Example 4. The support is

then transfered to a column (0.8 x 20 cm) which is eluted at 3 °C by 40 ml of 0.25 mmol.l^{-1} adenosine in 0.75 mol.l^{-1} potassium chloride containing 1 mmol.l^{-1} dithiothreitol. The effluent is chromatographed on a column (1.20 x 60 cm) of Sephadex G-25 in 0.01 mol.l^{-1} potassium hydrogenphosphate, pH 7.4, containing 1 mmol.l^{-1} dithiothreitol. The enzymatic activity of the fractions (2 ml) is assayed as described under Example 6. The fractions containing enzymatic activity are pooled and glycerol is added to the final concentration of 20% (by vol.). The yield is 238 µg of electrophoretically pure SAH-hydrolase protein of specific activity 72.7 ncat.mg^{-1}.

Example 6

The enzymatic activity of SAH-hydrolase is determined in 100 µl of a solution which is 0.1 mol.l^{-1} in potassium hydrogenphosphate, pH 7.37, 0.003 mol.l^{-1} in dithiothreitol, 3.75 x 10^{-3} mol.l^{-1} in L-homocysteine, and 2.5 x 10^{-5} mol.l^{-1} in adenosine. The solution assayed (50 µl) is added, the mixture is incubated 10 min at 37 °C, and a 10- µl sample of the mixture is separated on a column of Separon® SI C18 (5 µ) (3.3 x 150 mm) eluted by 0.01 mol.l^{-1} potassium dihydrogenphosphate, pH 2.8, containing 10 volume per cent of methanol. The flow rate is 0.4 ml/min, the effluent is monitored at 254 nm. The elution profile is continuously recorded in a recording UV-detector and the peak areas of adenosine and S-adenosyl-L-homocysteine, whose positions are determined in advance in runs with standards, are evaluated by planimetry. The number of enzyme activity units is calculated from the formula

$$EU = 0.5 \times 10^{-5} K$$

where 1 EU is the quantity of enzyme converting 1 umol of substrate in 1 min and K the conversion of adenosine into S-adenosyl-L-homocysteine expressed in per cent. This value corresponds to the quantity of enzyme in 50 µl of the mixture assayed.

Table 1 Preparation and properties of 9-(hydroxyalkyl)-8-bromoadenines of formula III [a]

Starting compound II (mmol)	R^1	R^2	R^3	n	Yield %	Method [b]	Melting point °C	R_f [c] S1	R_f [c] S2
30.0	H	-	H	0	58	A	238-239	0.76	0.72
10.0	H	H	H	1	62	B	205-207	0.59	0.63
40.0	H	OH	H	1	92	A	260	0.63	0.50
6.0	H	OH	CH_3	1	75	B	224	0.72	0.68
2.5	H	OH	CH_2OH	1	95	B	260	0.60	0.46
15.0	H	-	CH_3	0	70	B	191-192	0.58	0.63
4.0	CH_2OH	-	H	0	50	A	235-236	0.68	0.57

Remarks: [a] Ultraviolet spectra of compounds III (pH 2 and 7): λ_{max} 267 nm (ε_{max} 18 000); [b] according to Example 1 (A); according to Example 2 (B); [c] 80% aqueous 2-propanol-conc. aqueous ammonia (9:1) (S1) (paper chromatography; chloroform-methanol (4:1) (S2) (silica gel thin layer).

Table 2 Preparation and properties of 9-(aminoalkyl)-8-hydroxyadenines of formula I [a], where X is H

Starting compound III (mmol)	R^1	R^2	R^3	n	Yield %	Melting point °C	R_F [b]	Mass spectrum (M^+) [c]
10.0	H	-	H	0	57	251-252	0.38	194
5.0	H	H	H	1	48	217-218	0.38	208
15.0	H	OH	H	1	63	174	0.30	224
2.5	H	OH	CH_3	1	54	168-170	0.46	238
2.5	H	OH	CH_2OH	1	42	149	0.35	254
7.5	H	-	CH_3	0	64	175-176	0.53	208
2.5	CH_2OH	-	H	0	56	145	0.40	224

Remarks: [a] Ultraviolet spectra of compounds of formula I, where X is H, pH 2, λ_{max} 270 nm (ε_{max} 13 000); pH 12, λ_{max} 280 nm (ε_{max} 14 000); [b] 80% aqueous 2-propanol-conc. aqueous ammonia (9:1) (paper chromatography); [c] molecular peak.

Table 3 Preparation of affinity supports of formula I, where X is $CO(CH_2)_6R^4$, and their properties (procedure described under Example 3)

No. of support	Compound of formula I where X is H				Number of equivalents (a)	Compound of formula IV						Total number of equivalents (b)	Content of bound compound of formula I (X = H) (μmol/ml) (c)
	R^1	R^2	R^3	n		R^5	R^6	R^7	R^8	m	Y		
1	H	-	H	0	2.5	CH_3	CH_3	CH_3	C_6H_{11}	3	Ts[d]	25	8.0
2	H	H	H	1	5.0	$(CH_2CH_2)_2O$	CH_3	C_6H_{11}		2	Cl	60	12.0
3	H	OH	H	1	2.5	CH_3	CH_3	CH_3	C_6H_{11}	3	Ts	25	10.0
4	H	-	CH_3	0	5.0	CH_3	CH_3	CH_3	C_6H_{11}	2	Cl	60	8.0
5	H	-	$CH(OH)CH_2OH$	0	2.5	CH_3	CH_3	CH_3	C_2H_5	3	Cl	25	10.0
6	H	-	$CHOHCH_3$	0	10.0	CH_3	CH_3	CH_3	C_6H_{11}	3	Ts	60	7.0
7	CH_2OH	-	H	0	2.5	$(CH_2CH_2)_2O$	C_2H_5	C_6H_{11}		2	Cl	30	7.0

(a) based on the capacity of the support (10-14 μmol/ml gel slurry); (b) the total number of equivalents is divided into two portions; based on the capacity of the starting dextran gel (10-14 μmol/ml gel slurry); (c) determined spectrophotometrically at 260 nm with suspension of compound I, where X is $CO(CH_3)_6R^4$, in 50% aqueous glycerol; (d) Ts stands for p-toluenesulfonate

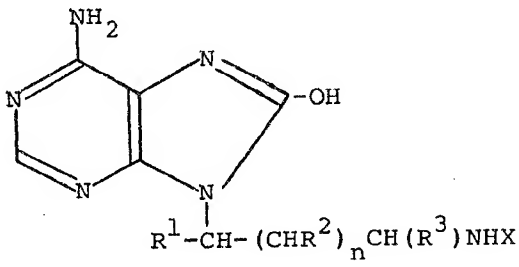
Table 4 Isolation of rat liver S-adenosyl-L-homocysteine hydrolase by affinity chromatography according to Example 4 (from 50 EU of enzymatic activity applied)

Support [a]	Losses due to nonspecific desorption (%)	Yield of pure enzyme (%)
1	2.5	32
2	0	39
3	0	40
4	15	29
7	0	33

[a] Designation according to Table 3

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

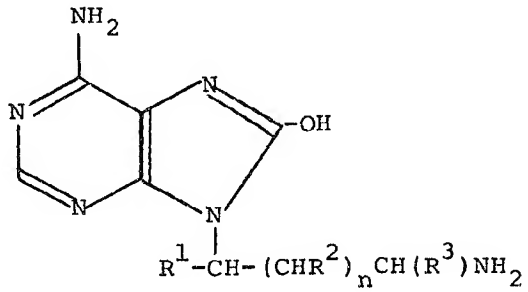
1. A compound of general formula (I)



(I)

where R^1 and R^3, independently, are a hydrogen atom, an alkyl group of 1 to 4 carbon atoms, or a hydroxyalkyl group with one to four carbons in the alkyl moiety, R^2 is a hydrogen atom or an hydroxy group, X is H or $CO(CH_2)_6R^4$ and R^4 is the residue of a macromolecular dextran gel, and n is 0 or 1.

2. A compound of general formula (Ia)

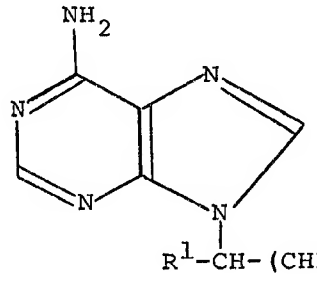


(Ia)

where R^1 and R^3, independently, are a hydrogen atom, an alkyl group of 1 to 4 carbon atoms, or a hydroxylalkyl group with one to four carbons in the alkyl moiety, R^2 is a

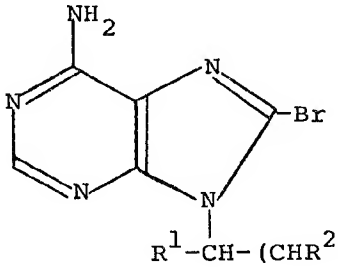
hydrogen atom or an hydroxy group, and n is 0 or 1.

3. Method of preparation of compounds of general formula (Ia) as defined in claim 2, according to which 9-(hydroxyalkyl)adenine of general formula (II)



(II)

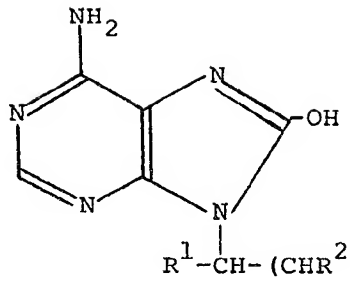
where R^1, R^2, R^3 and n are as defined above, is made to react with bromine in aqueous solution or suspension, at a temperature of 0 to 30°C and the 9-(hydroxyalkyl)-8-bromo-adenine of general formula (III)



(III)

where R^1, R^2, R^3 and n are as defined above is heated at a temperature of 80 to 150°C with an aqueous 20 to 30% (by weight) solution of ammonia at a weight ratio of 1:10 to 1:40.

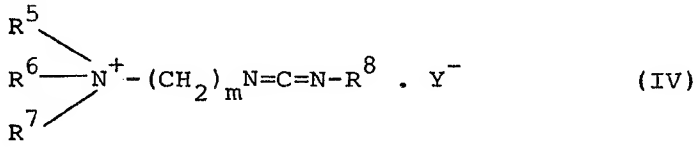
4. A compound of general formula (Ib)



(Ib)

where R^1 and R^3, independently, are a hydrogen atom, an alkyl group of 1 to 4 carbon atoms, or a hydroxyalkyl group with one to four carbons in the alkyl moiety, R^2 is a hydrogen atom or an hydroxy group, X is $CO(CH_2)_6R^4$ and R^4 is the residue of a macromolecular dextran gel, and n is 0 or 1.

5. Method of preparation of a compound of general formula (Ib) as defined in claim 4, according to which a dextran gel with 6-carboxyhexyl groups, is made to react with a 9-aminoalkyl-8-hydroxyadenine of general formula (Ia) as defined in claim 2, at a ratio of 1 to 10 molar equivalents in terms of carboxyl groups of the dextran gel, and in the presence of one or more salts of soluble carbodiimides of general formula (IV)

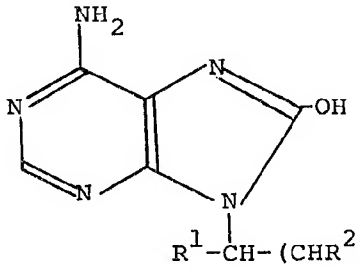


(IV)

where R^5, R^6 and R^7 are methyl groups, or R^5 and R^6 together are $(-CH_2CH_2)_2O$ and R^7 is a methyl or ethyl group, R^8 is a cyclohexyl or ethyl group, Y^- is a chloride or p-toluenesulfonate anion and m is 2 to 4, in aqueous solution at pH 5 to 6 and temperatures of 0 to 30°C; after completion of the reaction the insoluble gel is filtered off, washed with water and then a neutral buffer solution.

6. A method for the isolation of S-adenosine-L-homocysteine hydrolases from extracts of biological materials, consisting in adsorption of the crude or partly purified extract of biological material batch-wise or in a column to an affinity support of general formula (Ib) as defined in claim 4, and after washing the support with buffered solutions of neutral electrolytes, in displacing the S-adenosyl-L-homocysteine hydrolases by a small volume of adenosine solutions 0.01 to 1.0 $mmol.l^{-1}$ in an aqueous buffer at temperatures of 0 to 20°C, and in subsequent removal of adenosine from the eluate by gel filtration or dialysis.

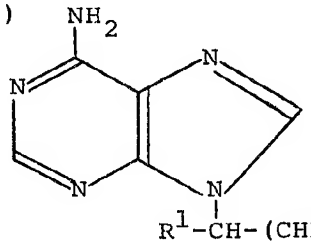
7. Method of preparation of a compound of general formula (I)



(I)

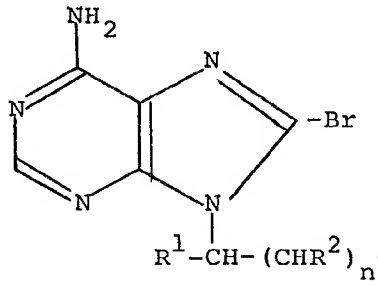
where R^1 and R^3, independently, are a hydrogen atom, an alkyl group of 1 to 4 carbon atoms, or a hydroxyalkyl group with one to four carbons in the alkyl moiety, R^2 is a hydrogen atom or an hydroxy group, X is H or $CO(CH_2)_6R^4$ and R^4 is the residue of a macromolecular dextran gel, and n is 0 or 1, characterized in that

(a) when X is H, a 9-(hydroxyalkyl)adenine of general formula (II)



(II)

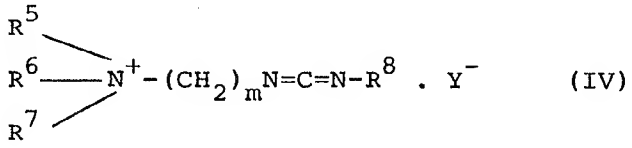
where R^1, R^2, R^3 and n are as defined above is made to react with bromine in aqueous solution or suspension, at a temperature of 0 to 30°C and the 9-(hydroxyalkyl)-8-bromoadenine of general formula (III)



(III)

where R^1, R^2, R^3 and n are as defined above is heated at a temperature of 80 to 150°C with an aqueous 20 to 30% (by weight) solution of ammonia at a weight ratio of 1:10 to 1:40, and

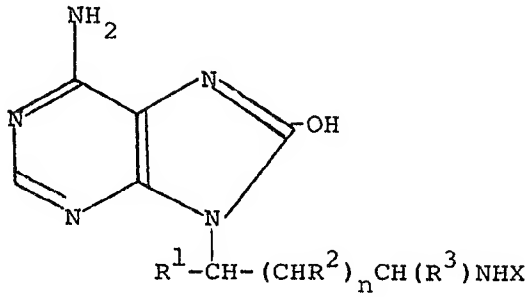
(b) when X is $CO(CH_2)_6R^4$, a dextran gel with 6-carboxyhexyl groups, is made to react with a 9-aminoalkyl-8-hydroxyadenine of general formula (I) where X is H at a ratio of 1 to 10 molar equivalents in terms of carboxyl groups of the dextran gel, and in the presence of one or more salts of soluble carbodiimides of general formula (IV)



(IV)

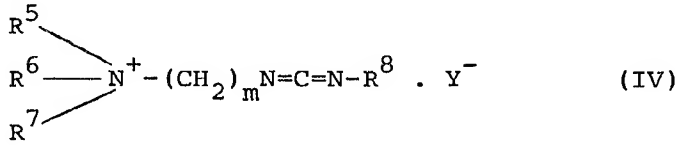
where R^5, R^6 and R^7 are methyl groups, or R^5 and R^6 together are $(-CH_2CH_2)_2O$ and R^7 is a methyl or ethyl group, R^8 is a cyclohexyl or ethyl group, Y^- is a chloride or p-toluene-sulfonate anion and m is 2 to 4, in aqueous solution at pH 5 to 6 and temperatures of 0 to 30°C; after completion of the reaction the insoluble gel is filtered off, washed with water and then a neutral buffer solution.

8. A compound of general formula (Ib)



where R^1 and R^3, independently, are a hydrogen atom, an alkyl group of 1 to 4 carbon atoms, or a hydroxyalkyl group with one to four carbons in the alkyl moiety, R^2 is a hydrogen atom or an hydroxy group, X is $CO(alk)R^4$, alk is a suitable alkylene chain and R^4 is the residue of a macromolecular dextran gel, and n is 0 or 1.

9. Method of preparation of a compound of general formula (Ib) as defined in claim 8, according to which a dextran gel with ω-carboxyalkyl groups, is made to react with a 9-aminoalkyl-8-hydroxyadenine of general formula (Ia) as defined in claim 2 at a ratio of 1 to 10 molar equivalents in terms of carboxyl groups of the dextran gel, and in the presence of one or more salts of soluble carbodiimides of general formula (IV)

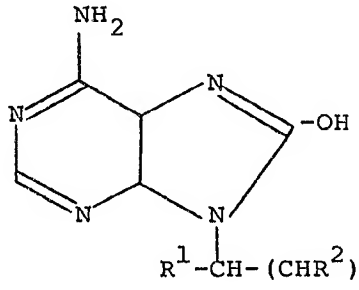


(IV)

where R^5, R^6 and R^7 are methyl groups, or R^5 and R^6 together are $(-CH_2CH_2)_2O$ and R^7 is a methyl or ethyl group, R^8 is a cyclohexyl or ethyl group, Y^- is a chloride or p-toluenesulfonate anion and m is 2 to 4, in aqueous solution at pH 5 to 6 and temperatures of 0 to 30°C; after completion of the

reaction the insoluble gel is filtered off, washed with water and then a neutral buffer solution.

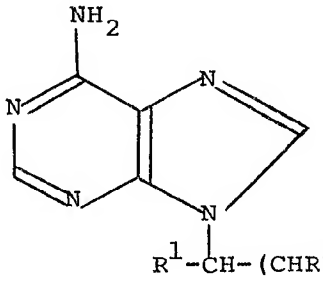
10. Method of preparation of a compound of general formula (I)



(I)

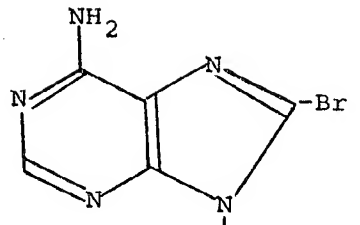
where R^1 and R^3, independently, are a hydrogen atom, an alkyl group of 1 to 4 carbon atoms, or a hydroxylalkyl group with one to four carbons in the alkyl moiety, R^2 is a hydrogen atom or an hydroxy group, X is a H or CO(alk)R^4, alk is a suitable alkylene chain, and R^4 is the residue of a macromolecular dextran gel, and n is 0 or 1, characterized in that

(a) when X is H, a 9-(hydroxyalkyl)adenine of general formula (II)



(II)

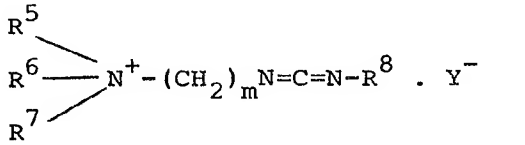
where R^1, R^2, R^3 and n are as defined above, is made to react with bromine in aqueous solution or suspension, at a temperature of 0 to 30°C and the 9-(hydroxyalkyl)-8-bromoadenine of general formula (III)



(III)

where R^1, R^2, R^3 and n are as defined above is heated at a temperature of 80 to 150°C with an aqueous 20 to 30% (by weight) solution of ammonia at a weight ratio of 1:10 to 1:40, and

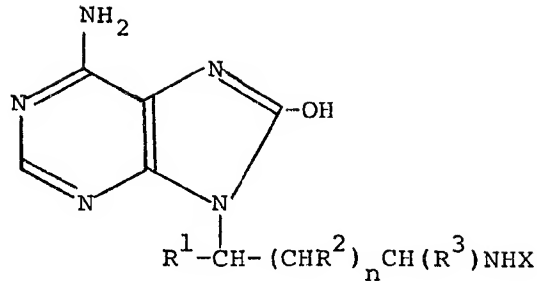
(b) when X is CO(alk) R^4, a dextran gel with ω-carboxyalkyl groups, is made to react with a 9-aminoalkyl-8-hydroxyadenine of general formula (I) where X is H at a ratio of 1 to 10 molar equivalents in terms of carboxyl groups of the dextran gel, and in the presence of one or more salts of soluble carbodiimides of general formula (IV)



(IV)

where R^5, R^6 and R^7 are methyl groups, or R^5 and R^6 together are $(-CH_2CH_2)_2O$ and R^7 is a methyl or ethyl group, R^8 is a cyclohexyl or ethyl group, Y^- is a chloride or p-toluene-sulfonate anion and m is 2 to 4, in aqueous solution at pH 5 to 6 and temperatures of 0 to 30°C; after completion of the reaction the insoluble gel is filtered off, washed with water and then a neutral buffer solution.

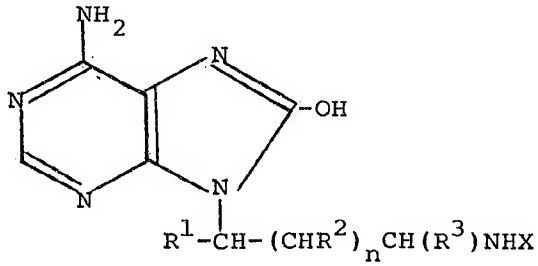
11. A compound of general formula (I)



(I)

where R^1 and R^3, independently are a hydrogen atom, an alkyl group of 1 to 4 carbon atoms, or a hydroxyalkyl group with one to four carbons in the alkyl moiety, R^2 is hydrogen or the hydroxy group, X is H or the residue of a suitable polymer support and n is 0 or 1.

12. A compound of general formula (I)



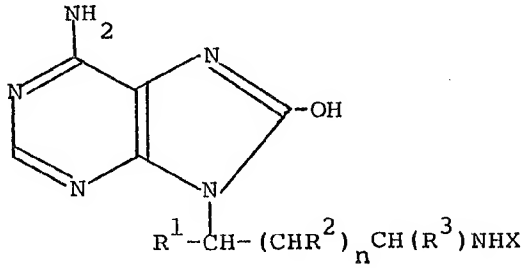
(I)

where R^1 and R^3, independently, are a hydrogen atom, an alkyl group of 1 to 4 carbon atoms, or a hydroxyalkyl group with one to four carbons in the alkyl moiety, R^2 is hydrogen or the hydroxy group, X is the residue of a suitable polymer support and n is 0 or 1.

13. A method for the isolation of S-adenosine-L-homocysteine hydrolases from extracts of biological materials, consisting in adsorption of the crude or partly purified extract of biological material batch-wise or in a column to an affinity support of general formula (I) as defined in

claim 12, and after washing the support with buffered solutions of neutral electrolytes, in displacing the S-adenosyl-L-homocysteine hydrolases by a small volume of adenosine solutions 0.01 to 1.0 $mmol.l^{-1}$ in an aqueous buffer at temperatures of 0 to 20°C, and in subsequent removal of adenosine from the eluate by gel filtration or dialysis.

14. A compound of general formula (I)



(I)

where R^1 and R^3, independently, are a hydrogen atom, an alkyl group or a hydroxyalkyl group, R^2 is a hydrogen atom or an hydroxy group, X is H or is the residue of a suitable dextran gel, sepharose or agarose support, and n is 0 or 1.

SUBSTITUTE

REMPLACEMENT

SECTION is not Present

Cette Section est Absente